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COMMUNICATIONS

The effect of dopamine and of apomorphine on dB-cAMP-induced stimulation of synaptosomal tyrosine hydroxylase

We have reported that dibutyryl cyclic AMP (dB-cAMP) stimulates the biosynthesis of [^{14}C]dopamine from [^{14}C]tyrosine in striatal slices and in synaptosomal preparations obtained from the striatum of rats (Goldstein, Anagnoste & Shirron, 1973; Roberge, Ebstein & Goldstein, 1974). The stimulation of [^{14}C]dopamine biosynthesis elicited by dB-cAMP occurs before, or at, the tyrosine hydroxylase step. The stimulation of tyrosine hydroxylase activity elicited by dB-cAMP might be due to permeability changes across the neuronal membrane, to an activation of tyrosine hydroxylase, or to an increase in levels of the cofactor. If the latter mechanism is responsible for the dB-cAMP-elicited stimulation of tyrosine hydroxylase activity, then the inhibition of tyrosine hydroxylase activity by the end-products should be diminished. We have therefore investigated the effects of dB-cAMP on the inhibition of tyrosine hydroxylase activity by dopamine. Since tyrosine hydroxylase activity is inhibited also by apomorphine (Goldstein, Freedman & Backstrom, 1970), a compound that stimulates dopamine receptors, we have investigated its effect on the stimulation of the enzyme activity elicited by dB-cAMP.

Male Sprague-Dawley rats, 200–250 g, were decapitated and the striata were immediately dissected. The tissue was homogenized in 10 volumes of ice-cold 0·32M sucrose using a homogenizer with a Teflon pestle. The homogenate was centrifuged at 1000 *g* for 10 min and the supernatant was used as a crude synaptosomal preparation. Tyrosine hydroxylase activity was measured in the synaptosomal preparation according to the procedure of Nagatsu, Levitt & Udenfriend (1964). The incubation mixture contained the following components: 100 μl striatal homogenate (approximately 10 mg of tissue), 100 μl of [^{3}H]-3,5-tyrosine (2×10^{-5}M, approximately 0·5 μCi) and 50–100 μl of test substances. All substances were dissolved in Krebs Ringer phosphate buffer, pH 7·2, and the final volume of the incubation mixture was adjusted with this buffer to 350 μl. The striatal homogenate was preincubated with the test substances for 5 or 10 min and the incubation was initiated by addition of the labelled tyrosine. The reaction mixture was incubated for 20 min at 37° and the reaction was stopped by addition of glacial acetic acid. The mixture was assayed as described by Nagatsu & others (1964). Tyrosine hydroxylase activity is expressed as pmoles of product formed per 10 mg synaptosomal preparation per 20 min.

Levels of significance were determined by calculating either the 95 or 99% confidence limits for each percentage (Goldstein, 1964). When the confidence limits of two values being compared did not overlap they were considered significantly different.

The effects of dopamine on striatal synaptosomal tyrosine hydroxylase activity are presented in Table 1. The enzyme activity was inhibited at 10^{-6}M dopamine by 32%. The inhibitory effectiveness of dopamine was reduced from 32 to 15% by the presence of dB-cAMP in the incubation medium. Correspondingly, the percentage of stimulation of tyrosine hydroxylation elicited by dB-cAMP was increased from 54 to 94% in the presence of 10^{-6}M dopamine in the incubation medium.

The effects of apomorphine on striatal synaptosomal tyrosine hydroxylase activity are presented in Table 2. The enzyme activity was inhibited at $6{\cdot}5 \times 10^{-7}$M apomorphine by 51% in synaptosomal preparations obtained from the striatum. The inhibitory effectiveness of apomorphine was reduced from 51 to 21% in the presence

Table 1. *The effect of dopamine on dB-cAMP-induced stimulation of tyrosine hydroxylase (T.H.) activity in synaptosomal preparations obtained from striatum of rats.* The values represent averages of results from six experiments ± s.d. The concentration of dB-cAMP was 10^{-3}M. Theophylline was added together with dB-cAMP and its concentration was 5×10^{-5}M.

Concentration of dopamine	Control		+ dB-cAMP		
	T.H. activity	% Inhibition by dopamine	T.H. activity	% Stimulation by dB-cAMP	% Inhibition by dopamine
0	105 ± 6·7	—	162 ± 7·8	54	—
10^{-6}M	71 ± 6·6	32	138 ± 3·9	94*	15**

* Value differs from the % stimulation in absence of dopamine, $P < 0{\cdot}05$.
** Value differs from % inhibition in controls, $P < 0{\cdot}05$.

of dB-cAMP in the incubation mixtures. Correspondingly, the percentage of stimulation of tyrosine hydroxylation elicited by dB-cAMP was increased from 59 to 160% in incubation mixtures with apomorphine. Haloperidol alone reduced slightly the synaptosomal tyrosine hydroxylase activity and partially reversed the inhibitory effectiveness of apomorphine. Thus, haloperidol, as well as dB-cAMP reduces the inhibitory effectiveness of apomorphine, but the effects of both compounds are not additive. The dB-cAMP-elicited stimulation of synaptosomal tyrosine hydroxylase activity was not affected by haloperidol alone. However, haloperidol partially reversed the enhanced stimulation of tyrosine hydroxylase activity elicited by db-cAMP in the presence of apomorphine.

Dopamine and apomorphine inhibit synaptosomal tyrosine hydroxylase activity and both enhance the dB-cAMP elicited stimulation of the enzyme activity. Apomorphine proved to be a more potent inhibitor of synaptosomal tyrosine hydroxylase activity than dopamine. This difference might either be due to deamination of

Table 2. *The effect of apomorphine and/or haloperidol on dB-cAMP-induced stimulation of tyrosine hydroxylase (T.H.) activity in synaptosomal preparations obtained from striatum of rats.* The values represent averages of results from five experiments ± s.d. The concentration of dB-cAMP and of theophylline is the same as indicated in Table 1. Haloperidol was added 5 min before the addition of apomorphine.

Concentration of tested drugs	Control		+ dB-cAMP		
	T.H. activity	% Inhibition	T.H. activity	% Stimulation by dB-cAMP	% Inhibition
—	107 ± 7·1	—	170 ± 21·8	59%	—
Haloperidol 2×10^{-7}M	93 ± 8·5	13%	148 ± 14·6	59%	13%
Apomorphine $6{\cdot}5 \times 10^{-7}$M	52 ± 2·8	51%	135 ± 7·4	160%*	21%**
Haloperidol 2×10^{-7}M + apomorphine $6{\cdot}5 \times 10^{-7}$M	72 ± 2·3	23%***	139 ± 8·0	93%****	6%

* Value differs from % stimulation in absence of any tested drugs, $P < 0{\cdot}01$.
** Value differs from % inhibition in controls, $P < 0{\cdot}01$.
*** Value differs from % inhibition in presence of apomorphine alone, $P < 0{\cdot}01$.
**** Value differs from % stimulation in presence of apomorphine alone, $P < 0{\cdot}01$.

dopamine by monoamine oxidase in the synaptosomal preparations or to the double action of apomorphine on the enzyme activity, one related to the inhibitory property of the catechol moiety and the other related to the activation of the presynaptic dopamine receptors. The results of this study and of a recently reported study (Christiansen & Squires, 1974) show that haloperidol partially reverses the inhibitory effectiveness of apomorphine, indicating that apomorphine exerts its action on synaptosomal tyrosine hydroxylase activity, at least partially, by stimulating the presynaptic dopamine receptors. It is of interest to note that compounds that stimulate dopamine receptors, such as S-584 (1-(3,4-dihydroxy benzyl)-4-(2-pyrimidinyl) piperazine), the catechol metabolite of Trivastal, and CB-154 (2-Br-α-ergokryptin) inhibit the synaptosomal tyrosine hydroxylase activity and enhance the dB-cAMP-elicited stimulation of the enzyme (Goldstein, Ebstein & Roberge, unpublished data). One is therefore tempted to propose that the inhibition of tyrosine hydroxylase activity and the enhancement of the dB-cAMP-elicited stimulation of the enzyme activity might be linked to the receptor stimulating activity of these agents.

Since the inhibition of tyrosine hydroxylase activity by catechols is competitive with the pteridine cofactor (Udenfriend, Zaltman-Nirenberg & Nagatsu, 1965), one would expect that the inhibition by dopamine and apomorphine would be dependent upon the concentration of the cofactor. The finding that dB-cAMP reduces the inhibitory effectiveness of dopamine and of apomorphine indicates that the cyclic nucleotide elicits the stimulation of synaptosomal tyrosine hydroxylase activity by increasing the concentration of the cofactor or by allosteric activation of tyrosine hydroxylase. Further studies are now in progress to determine whether the former or the latter mechanism is responsible for the dB-cAMP-elicited stimulation of tyrosine hydroxylase activity.

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REFERENCES

CHRISTIANSEN, J. & SQUIRES, R. F. (1974). *J. Pharm. Pharmac.*, **26**, 367–369.

GOLDSTEIN, A. (1964). In: *Biostatistics: An Introductory Text.* pp. 184–187. N.Y.: McMillan.

GOLDSTEIN, M., ANAGNOSTE, B. & SHIRRON, C. (1973). *J. Pharm. Pharmac.*, **25**, 348–351.

GOLDSTEIN, M., FREEDMAN, L. S. & BACKSTROM, T. (1970). *Ibid.*, **22**, 715–717.

NAGATSU, T., LEVITT, M. & UDENFRIEND, S. (1964). *Analyt. Biochem.*, **9**, 122–126.

ROBERGE, C., EBSTEIN, B. & GOLDSTEIN, M. (1974). *Fedn Proc. Fedn Am. Socs exp. Biol.*, **33**, No. 3. Abstr. No. 1751.

UDENFRIEND, S., ZALTZMAN-NIRENBERG, P. & NAGATSU, T. (1965). *Biochem. Pharmac.*, **14**, 837–845.